PROTOCOL FOR SUBRENAL IMPLANT AND CHEMOTHERAPY

OF THE MCF-7/ADR BREAST XENOGRAFT

MODEL: (3KDG5) Subrenal Capsule MCF-7/ADR Breast Xenograft (delayed treatment).

Origin of Tumor Line: (No details).

Summary of Test Procedures: A tumor fragment is implanted under the membranous covering of the kidney of either athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCr-nu) mice. IP test agent treatment starts 4 days after tumor implant and is repeated every fourth day for a total of 4 injections. The parameter is change in tumor weight.

ANIMALS: (refer to Protocol 8).

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCr-nu) mice.

Weight: Mice should have a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

XPERIMENT SIZE:

General Testing: Six animals per test group and 10 animals per control group and 10 animals for an early control group. Typically, a single test agent experiment is run with 4 dose levels of the test agent at 50% intervals. Total number of mice in a typical single test agent experiment (tests, control, and early control) is 44.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6).

PROPAGATION

Fragment: Prepare a 2x2x2 mm fragment of s.c. donor tumor.

Time: When donor tumor reaches 200-400 mg (approximately Day 28 after implant).

Site: Implant fragment s.c. into axillary region with puncture in inguinal region using a 13-gauge trocar.

TESTING

Fragment: Prepare a 19x19x19 Ocular Micrometer Unit (OMU) fragment. Average diameter must be 17-21 OMU's measured under a dissecting microscope.
10 OMU's = 1 mm.

Anesthetic: Any satisfactory anesthetic (e.g., chloral hydrate, Avertin, etc).

Medium: Tissue culture medium with no antibiotics (e.g., 199, Eagles MEM, or Earles).

Time: When donor tumor reaches 200-400 mg (approximately Day 28 after implant).

Site: Implant fragment under the subrenal capsule using a 16-gauge trocar with a 22° bevel, after exposing the kidney with a 7-mm dorsal skin incision. The wound is closed with a 9-mm wound clip after closing the peritoneum with 1-4 silk sutures.

TESTING SCHEDULE: (refer to Protocols 3 and 4).

Day 0: Anesthetize animals. Implant tumor and measure. Randomize animals after they recover from the anesthetic. Run bacterial cultures (refer to Protocol 7). Determine solubilities of test agent. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated.

Day 2: Recheck cultures. Discontinue if contaminated and report accordingly.

Day 4: Record body weights (Weigh Day 1). Sacrifice <u>early control</u> group, measure tumors in OMU's. Calculate mean tumor weight. Record this mean tumor measurement as initial day (Day 4) tumor measurement for <u>control group and all test groups</u>. In order to use the early control measurements for the initial measurements of the treated groups, it will be necessary to reduce the number from 10 to 6. Do this by eliminating the two largest and the two smallest

measurements of the early control group. Prepare test materials. Initiate ip test agent injections based on individual body weight. Treatment is q4d on Days 4, 8, 12, and 16. Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Days 8,12,16: Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 19: End and evaluate experiment. Record body weights (Weigh Day 2). Measure tumors in OMU's and record. Final evaluation day for this model is also test toxicity day, test no-take day, control early death day, control no-take day and Weigh Day 2.

QUALITY CONTROL:

(1) Positive control compound for this tumor system has not been established as of this publication date.

(2) Implant 2 or 3 additional mice which can be used for replacements in the event of surgical deaths. If surgical deaths do not occur, use these mice as additional control animals.

(3) Within a given experiment, whenever possible, use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.

(4) House mice 3 to 6 per cage.

(5) Donor tumor should weigh between 200-400 mg and be scrupulously cleaned of necrotic and/or hemorrhagic areas.

(6) In case of unusual deaths, these animals should be autopsied and peculiarities noted.

(7) Specific definitions for subrenal capsule implants for Control Status Code assignments by the computer (refer to Protocol 7.7) are:

(a) Acceptable control mean tumor weight change is $\geq$20% between Day 4 and Final Evaluation Day.

(b) Control no-take: A mouse with a tumor weight increase of <20% between Day 4 and Final Evaluation Day (computer determined).

(c) Excessive control no-takes: 2 or more no-takes are excessive in a control group of 10 mice (refer to Protocol 7.3).

(d) Excessive control deaths: 2 or more control deaths in a group of 10 to 19 animals (i.e., >10%) on or before Final Evaluation Day.

EVALUATION: (refer to Protocol 11).

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Compute mean animal body weights for Day 4 and Day 19, compute T/C for all test groups with >65% survivors on Day 19. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

The NCI screening laboratories on Day 4 (measurements obtained from early control group, Day 4) and on Final Evaluation Day are to measure and input OMU length and width measurements for tumors. The dimensions are measured and recorded in OMU's. (They will be entered on the WS 180 Solid Tumor Data Form using type 2 with code H, per instructions of 9/81 from the Screener Instructions for Use of the Solid Tumor Input Form, Section 3.3.1.2. By convention, the length (L) dimension must be entered first). The NCI computer:

(1) Converts OMU's to millimeters (mm).

(2) Calculates tumor weights (mgs) from tumor dimensions (mm x mm) following the formula for a prolate ellipsoid:

$$\frac{L \times W^2}{2}$$ Where L is the longer of the two measurements

(3) Calculates the change (delta) in mean tumor weight for each group of mice:

Change in Mean Tumor Weight =

Mean Tumor Weight $_{FINAL}$ - Mean Tumor Weight $_{INITIAL\ (Day\ 4)}$

(4) Calculates the change (delta) in mean tumor weight for tests (T) and control (C) groups.

(5) Calculates T/C% for all test groups with >65% survivors on Final Evaluation Day:

$$T/C\% = \frac{\triangle WtT}{\triangle WtC} \times 100 \text{ -- if } \triangle WtT \text{ positive.}$$

$$T/C\% = \frac{\triangle WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL (Day 4)}}} \times 100 \text{ -- if } \triangle WtT \text{ Negative}$$

CRITERIA FOR ACTIVITY:

An initial T/C $\leq$20% is considered necessary to demonstrate moderate activity.

A reproducible T/C $\leq$ 0% is considered significant activity.

REPORTING OF DATA: On the final day of testing, prepare final control and test reports. Input data. Screener assigns a code of "U" to an individual mouse whose response screener considers invalid, including the following circumstances:

(1) Tumor lost from site of implant and kidney appears normal.

(2) Animal dies or appears ill and loses weight -- not attributable to test agent toxicity -- for any reason, including fighting.

(3) More than one tumor present.

(4) Infection at site of implant.

(5) Kidney does not appear normal.

A comment must accompany all "U" code designations. The screener designates as unsatisfactory (assigns a Test Status Code of "33") all test groups that the screener considers invalid for any reason, including a case where more than 33% of the mice have been assigned a "U" code. The computer designates as unsatisfactory (assigns a Test Status Code of "34") all tests where:

(1) There is no control delta calculated.

(2) More than 33% of test mice have been assigned a "U" code.

(3) Less than 67% of "Non-U" test mice are acceptable for calculations (i.e., initial tumor diameters are between 17 and 21 OMU's and final measurements exists for Final Evaluation Day).

(4) Test groups where the control group contains $\geq$10% spontaneous tumor regressions.

A comment must be provided stating the reason for a TSC of "33", when a nonstandard dose is administered (whether due to a solubility problem or special request), and for poor suspensions.

The computer assigns the appropriate Control Status Code to reflect the acceptability of the control group, using definitions listed under Protocol 7 (Tumor Quality Control) including the codes cited in Section 7.7 and Instruction 14.